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(71) Applicant *(for all designated States except US)*: DE MONTFORT UNIVERSITY [GB/GB]; The Gateway, Leicester, Leicestershire LE1 9BH (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: POTTER, Gerard, Andrew [GB/GB]; 122 St. Leonards Road, Leicester, Leicestershire LE2 1WR (GB). PATTERSON, Lawrence, Hylton [GB/GB]; Gynsill Court, Flat 1, Gynsill Lane, Anstey, Leicester, Leicestershire LE3 7AH (GB). BURKE, Michael, Danny [GB/GB]; One Ash, 97 Uppingham Road, Houghton on the Hill, Leicester, Leicestershire LE7 9HL (GB). BUTLER, Paul, Crispin [GB/GB]; 12 Conway Road, Leicester, Leicestershire LE2 1PD (GB).

(74) Agent: McNEIGHT & LAWRENCE; Regent House, Heaton Lane, Stockport, Cheshire SK4 1BS (GB).

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(54) Title: HYDROXYLATION ACTIVATED PRODRUGS

(57) Abstract

The present invention concerns enzymatic aromatic hydroxylation–activated prodrugs, particularly anti–tumour prodrugs and those which are specifically activated by the hydroxylation activity of the enzyme CYP1B1.

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# Hydroxylation Activated Prodrugs

The present invention concerns enzymatic aromatic hydroxylation-activated prodrugs, particularly anti-tumour prodrugs and those which are specifically activated by the hydroxylation activity of the enzyme CYP1B1.

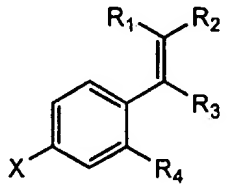
Many conventional cytotoxic drugs are known which can be used for chemotherapeutic purposes. However, they typically suffer from the problem that they are generally cytotoxic and therefore may affect cells other than those which it is wished to destroy. This can be alleviated somewhat by using targetted drug delivery systems, for example direct injection to a site of tumourous tissue, or by e.g. binding the cytotoxic agent to antibody which specifically recognises an antigen displayed by cancerous cells. Alternatively, electromagnetic radiation may be used to cause chemical changes in an agent at a desired site in the body such that it becomes cytotoxic. However, all of these techniques have, to a greater or lesser extent, certain limitations and disadvantages.

It has been reported (Murray, G.I. *et al.*, 15 July 1997, Cancer Research, 57: 3026-3031) that the enzyme CYP1B1, a member of the cytochrome P450 family of xenobiotic metabolizing enzymes, is expressed at a high frequency in a range of human cancers including cancers of the breast, colon, lung, oesophagus, skin, lymph node, brain and testis, and that it is not detectable in normal tissues. This led to the conclusion (p. 3030, final sentence) that "...the expression of CYP1B1 in tumour cells provides a molecular target for the development of new anticancer drugs that could be selectively activated by the presence of CYP1B1 in tumour cells". No specific anticancer drugs are suggested.

The present inventors have now succeeded in creating a range of prodrugs which have little or negligible cytotoxic effect when in their normal state, but which are highly cytotoxic (i.e. have a substantially increased cytotoxicity) when hydroxylated by CYP1B1. This provides for a self-targetting drug delivery system in which a non-cytotoxic (or at leat negligibly cytotoxic) compound can be administered to a patient, for example in a systemic manner, the compound then being hydroxylated at the site of tumour cells (intratumoural hydroxylation) to form a highly cytotoxic compound which acts to kill the tumour cells. The fact that CYP1B1 is not expressed by normal cells means that the hydroxylation of the compound only occurs at the site of tumour cells and therefore only tumour cells are affected, thus providing a self-targetting drug delivery system.

The prodrugs of the present invention have the distinct advantage of being useful in the treatment of tumours at any site in the body, meaning that even tumours which have undergone metastasis (which are not normally susceptible to site-specific therapies) may be treated, as well of course as primary and secondary tumours.

According to the present invention there is provided a prodrug activated by enzymatic aromatic hydroxylation and having the formula (I):




wherein:

X = H, OH or OMe;

$R_1$ = H, $C_{1-4}$ lower alkyl, CN or Ar;

$R_2$ = H, CN, $CONH_2$, $CSNH_2$, COAr or Ar; and

Ar = phenyl, pyridyl or substituted aryl;

and:

$R_3$ = H or $C_{1-4}$ lower alkyl; and

$R_4$ = H, OH or OMe;

or:

$R_3$,$R_4$ = $(CH_2)_n$, n=2, 3 or 4

The prodrug may be an anti-tumour prodrug. Examples of tumours include cancers (malignant neoplasms) as well as other neoplasms e.g. "innocent" tumours. The prodrug may be activated by hydroxylation by CYP1B1.

These prodrugs are styrene- or calchone-derivatives and their specific anti-tumour use is neither suggested nor disclosed by Murray, G.I. *et al.* (*supra*), nor is the fact that they are in fact prodrugs having an "activated" hydroxylated form. Where compounds of formula (I) have been previously identified and made, they have not been identified as anti-tumour agents due to their poor (or negligible) cytotoxicity. Thus the intratumoural hydroxylation of the prodrugs of the present invention provides them with a surprising and unexpected efficacy.

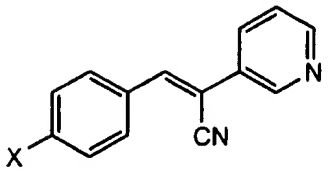
The styrene sub-structure of the compounds of formula (I) is essential in providing their efficacy. The Ar group may, for example, be a substituted aryl comprising 4-methoxyphenyl, 4-nitrophenyl, 3,5-dihydroxyphenyl or 3,4,5-trimethoxyphenyl, although other substituted aryls are, of course, also possible.

X may be hydroxy or methoxy.

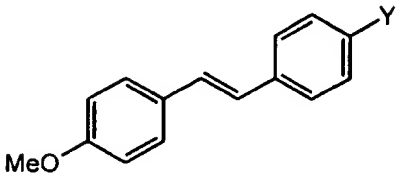
As specified in formula (I) $R_3$ an $R_4$ may together form an alkyl chain having 2-4 carbon atoms, and thus may form part of a cycloalkyl group having 5,6 or 7 carbon atoms.

The prodrug may have the formula of any one of formulae (II) - (V):

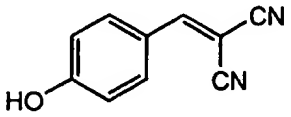
(II):




(III):




(IV):

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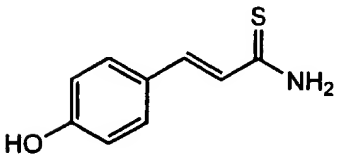
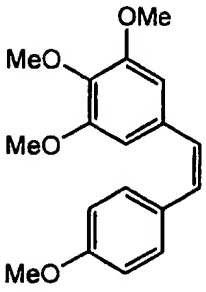


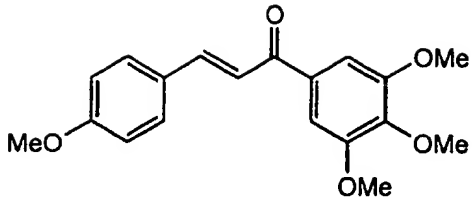

wherein X = OMe or OH, and Y = $NO_2$ or OMe.

Alternatively, the prodrug may have the formula of either one of formulae (VI) or (VII):

(VI):

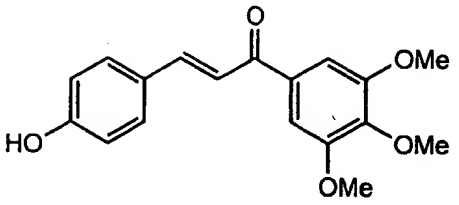


(VII):

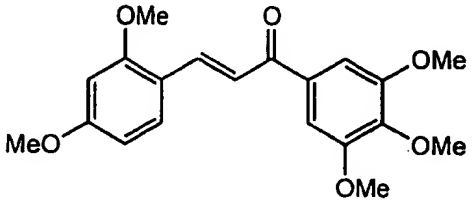



Alternatively the prodrug may have the formula of any one of formulae (VIII) - (XII):

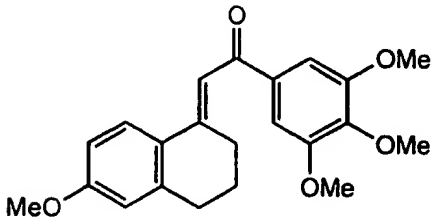
(VIII):



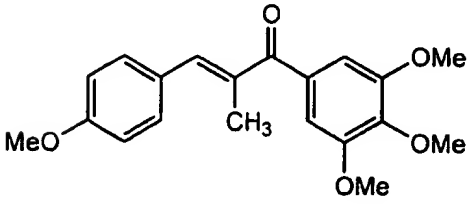

(IX):



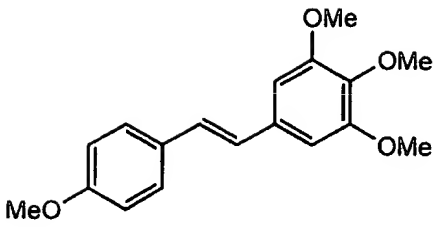

(X):




(XI):



(XII):




Hydroxylated forms of compounds (II) - (V) are potent tyrosine kinase inhibitors, and hydroxylated forms of compounds (VI) and (VII) are potent antimitotic agents. Previously, tyrosine kinase inhibitors have been of little chemotherapeutic benefit since the tyrosine kinase enzymes are ubiquitous in both normal and tumour cells and are thus not in themselves tumour-specific. However, the targetted production of tyrosine kinase inhibitor in tumour cells means that the inhibitory action will be specific to tumour cells. Furthermore, since the inhibitory activity will only be found in tumour cells, the tyrosine kinase inhibitor itself need not be isoform specific for a particular tyrosine kinase enzyme since any inhibition of tyrosine kinase activity will contribute to tumour inhibition and cell destruction.

Similarly, the antimitotic prodrugs of formulae (VI) and (VII) and (VIII) - (XII) are particularly useful since present antimitotic agents are of limited use due to the severe side-effects resulting from the poisoning of both normal and tumour cells. The present invention however allows for the specific *in situ* generation of the antimitotic agent at tumour cells, resulting in their specific targetting.

Methods of synthesis of the prodrugs of the present invention will be readily apparent to one skilled in the art, for example as exemplified below. The compounds of the invention may be prepared in a variety of different ways, for example by aldol condensation (Vogels Textbook of Practical Organic Chemistry, 4th Edition, p.146), by McMurry coupling (McMurry and Fleming, 1974, J. Am. Chem. Soc., 96: 4708-4709), or by the Wittig reaction (1973, Org. Synth. Coll., 5: 751).

Also provided according to the present invention is a prodrug according to the present invention for use in a method of treatment or diagnosis of the human or animal body, particularly the treatment or diagnosis of tumours.

Also provided according to the present invention is the use of a prodrug according to the present invention in the manufacture of a medicament for the treatment of tumours.

Also provided according to the present invention is a method of manufacture of a medicament, comprising the use of a prodrug according to the present invention. The medicament may be for the treatment of a tumour.

Also provided according to the present invention is a method of treatment or diagnosis of a tumour in a patient, comprising administering to the patient a prodrug according to the present invention.

Methods of manufacture of medicaments are well known. For example a medicament may additionally comprise a pharmaceutically acceptable carrier, diluant or excipient (Reminton's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA).

The exact dose (i.e. a pharmaceutically acceptable dose) of prodrug to be administered to a patient may be readily determined by one skilled in the art, for example by the use of simple dose-response experiments.

Since the prodrugs of the present invention are specific to tumour cells, they may not only be used to treat tumours, but may also be used to determine whether or not a patient (or a sample taken from a patient) has tumour cells. For example, cell numbers

in a sample may be assayed, as may the presence and quantity of the hydroxylated prodrug, thus providing for the diagnosis of the presence of tumour cells.

Also provided according to the present invention is the hydroxylated form of a prodrug according to the present invention.

The invention will be further apparent from the following description, which shows, by way of example only, forms of prodrugs.

The Figure shows the results of a 40 hour exposure of cells to the compound (VII) (also referred to as DMU 102). X-axis shows the concentration in μM of DMU 102. Y-axis shows the survival rate for cells, as a percentage of surviving cells in a control experiment. Error bars shows results ±1 SE (standard error). Circular markers are for cell line V79mz. Triangular markers are for cell line V79h1B1.

## EXPERIMENTAL

Prodrugs according to the present invention were synthesised as described below and the products of their hydroxylated metabolites assayed for the presence of the desired hydroxylation products. Their *in vitro* cytotoxicity against control and test cell lines was also determined.

### Microsomal preparation of resected human tumour tissue

A microsomal preparation of human tumour tissue expressing the CYP1B1 enzyme was prepared essentially as described by the method of Barrie *et al.* (1989, J. Steroid Biochem., 6: 1191-1195)

### Metabolism Studies

Experiment were carried out at 37 °C, under yellow light.

An array of 1.5 ml centrifuge tubes were set up in a water bath shaker under aerobic conditions. To each tube was then added 500 µl of pH 7.6 buffer (0.1 M $NaK_2PO_4$), followed by NADPH (5 µl of a 25 mM stock solution). The microsomal preparation (80 µl) was then added and the tubes preincubated for 5 minutes at 37 °C. The prodrug substrate was then added (10 µl of a 5 mM stock solution) and incubated for 1 hour at 37 °C. After 1 hour the tubes were transferred to an ice/water cooling bath (0 °C). The tubes were then centrifuged at 15,000 rpm for 30 minutes. A sample of the supernatant (100 µl) was then taken and analysed by HPLC.

HPLC conditions: Spherisorb C18 (25 cm x 4.6 mm id), used without guard column. Flow rate 1ml/min. Eluent 75% 0.1 M $KH_2PO_4$ and 25% acetonitrile.

The prodrugs were assayed as described above and were found to undergo aromatic hydroxylation. The hydroxylated metabolite was detected by HPLC, and confirmed by synthesis of the authentic hydroxylated metabolite.

Compound IIa (below), (Z)-1-Cyano-1-(3-pyridyl)-2-(4-methoxyphenyl)ethene, was converted to the hydroxylated metabolite (Z)-1-Cyano-1-(3-pyridyl)-2-(3-hydroxy-4-methoxyphenyl)ethene.

Compound IIIc, (E)-(3,4',5)-trihydroxystilbene was converted to the hydroxylated metabolite (E)-(3,3',4,5')-tetrahydroxystilbene.

Compound VII (E)-1-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one was converted to the hydroxylated metabolite (E)-1-(3-Hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one.

*In vitro* Cytotoxicity Studies

The cytotoxicity assay method used was a modification of the MTT cytotoxicity assay (Carmichael *et al.*, 1987, Cancer Research, 47: 936). The activity of the compounds were evaluated in cell lines which express the enzyme CYP1B1 (V79mzhu1B1) and the corresponding parental cell line which does not express CYP1B1 (V79mz) (Luch *et al.*, 1998, Chem. Res. Toxicol., 11: 686). $10^3$ cells were plated in 100µl DMEM (high glucose) (Dulbecco's Modified Eagles Medium, Life Science International) plus 10% heat-inactivated FBS (Foetal Bovine Serum. Hybrimax, Sigma.) *per* well of 96 well (Nunc) microtitre plates for 24 hours to allow adherance and metabolic recovery followed by addition in quadruplicate of compound at double strength in the same medium in 100 µl to give a final maximal concentration of 0.2% DMSO (dimethyl sulfoxide). Compound stocks were made up as 100 mM in DMSO and stored for no more than one month at 4 °C. The plates were then incubated at 37 °C, 5% $CO_2$, 100%

humidity for a further 48 hours followed by washing by immersion 3 times in Dulbecco's PBS (phosphate buffered saline) A. 50 µl of RPMI 1640 w/o phenol red (Roswell Park Memorial Institute Medium 1640, Life Science International) with 2 mg/ml MTT was then added for four hours as above, excess MTT removed by aspiration and 125 µl of DMSO added on a vortex for 30 minutes to solubilize the product. The absorbance at $A_{450}$ was recorded and the results expressed as a % survival of carrier only treated controls. From this data was calculated the IC50 value, which is the concentration at which 50% cytotoxicity is observed. Confirmation of expression of CYP1B1 was determined by immunocytology, Western blotting and EROD assay (ethoxyresorufin-O-dealkylase assay; Burke, M.D. *et al.*, 1985, Biochem. Pharmacol., 34: 3337) of cells used in the assay at the time point when the compounds were added, either fixed in methanol at -20 °C, or harvested from replicate plates and stored at -80 °C until assay.

The prodrugs were evaluated using the above assay system, and the results are shown in Table 1. These results demonstrate that the compounds of this invention exhibit differential toxicity against the CYP1B1 expressing cell line.

Table 1: Growth Inhibition of Cells not Expressing and Expressing CYP1B1 ($IC_{50}$ / uM ± 2 %)

| Compound | DMU Code No. | V79 Cells | V791B1 Cells |
|---|---|---|---|
| Compound IIa | DMU-201 | 1.81 | 1.63 |
| Compound IIIb | DMU-205 | 58.2 | 8.3 |
| Compound VII | DMU-102 | 1.0 | 0.005 |
| Compound VIII | DMU-116 | 1.81 | 1.71 |

Of particular note is the discovery that compound VII is around 200-fold more cytotoxic to the cell line expressing CYP1B1 than to the parental cell line not expressing this enzyme. Therefore compound VII is particularly useful as a tumour selective anticancer

agent. A plot of % cell survival versus concentration for compound VII (DMU-102) is shown in Figure 1.

Methods of Synthesis

Compound IIa

(Z)-1-Cyano-1-(3-pyridyl)-2-(4-methoxyphenyl)ethene (DMU-201)

To a stirred mixture of 4-methoxybenzaldehyde (2g, 14.69 mmol) and 3-pyridylacetonitrile (1.58 ml, 14.84 mmol) in methanol (30 ml) was added 50% w/v sodium hydroxide (1 ml). The reaction was stirred for 3 hours. The reaction mixture was quenched with water (20 ml), acidified with 2N HCl, then rebasified with dilute NaOH (aq), and the reaction product was extracted successively into dichloromethene (3 x 20 ml). The organic solutions were dried over anhydrous $MgSO_4$ and the solvent removed. Purification by column chromatography ($SiO_2$, Hexane/Ethylacetate 8:2; 1:1) gave 2.01g (58% yield) of the title compound as a straw coloured solid: 1H-NMR (CDCl3) 8.80 (d,1H), 8.50 (m,1H), 7.80 (m,3H), 7.45 (s,1H), 7.25 (m,1H), 6.90 (m,2H), 3.80 (s,3H). 13C NMR (CDCl3) 161.9, 157.6, 157.5, 149.5, 146.9, 143.3, 133.1, 131.4, 130.9, 126, 123.5, 117.7, 114.5, 105.2, 55.4. Mass Spectrum m/e (M+1) 237.

Hydroxylated Metabolite of Compound IIa

(Z)-1-Cyano-1-(3-pyridyl)-2-(3-hydroxy-4-methoxyphenyl)ethene (DMU-202)

A mixture of 4-methoxy-3-hydroxybenzaldehyde (0.5g, 3.3 mmoles), 3-pyridylacetonitrile (0.35 ml, 3.3 mmoles) and 50% w/v of aqueous NaOH (3 ml) in methanol (10 ml) was stirred at room temperature for 30 minutes. The yellow solid that precipitated was filtered, washed with cooled methanol (1 ml), cooled $CH_2Cl_2$ (5 ml) and dried under vacuum over $P_2O_5$ to yield 0.5g (60%) of the title compound as a yellow solid. 1H-NMR (CD3OD) 8.8 (m,1H), 8.5 (m,1H), 8.1 (m,1H), 7.7 (s, 1H), 7.5 (m,1H), 7.3 (d,1H), 7.2 (d,1H), 6.8 (d,1H). Mass Spectrum m/e (M+1) 253.

Compound IIb

(Z)-1-Cyano-1-(3-pyridyl)-2-(4-hydroxyphenyl)ethene (DMU-208)

A mixture of 4-hydroxybenzaldehyde (0.5g, 4.1 mmoles), 3-pyridylacetonitrile (0.54 ml, 4.1 mmoles) and 50% w/v of aqueous NaOH (3.3 ml) in methanol (10 ml) was stirred at room temperature for 30 minutes. The yellow precipitate that formed was filtered, washed with cooled methanol (1 ml), cooled $CH_2Cl_2$ (10 ml), and dried under vacuum over $P_2O_5$ to yield 0.6g (66%) of the title compound. 1H-NMR (CD3OD) 8.8 (d,1H), 8.4 (m,1H), 8.05 (m,1H), 7.8 (m,2H), 7.6 (s,1H), 7.4 (m,1H), 6.6 (m,2H). 13C-NMR (DMSO) 177.52, 175.57, 146.59, 145.06, 144.78, 133.15, 132.73, 130.97, 123.8, 120.8, 120.3, 114.3, 88.5; Mass Spectrum m/e (M+1) 223.

Compound IIIb (via McMurry Coupling)

(E)-(4,4')-Dimethoxystilbene (DMU-205)

LiAlH4 (0.5g, 13.18 mmoles) was added to a stirred slurry of $TiCl_3$ (3.13g, 26.35 mmol) under $N_2$ in dry THF (20 ml). An instantaneous reaction occurred, accompanied by the evolution of heat and gas and by a rapid change of colour to deep black. A THF solution of 4-methoxybenzaldehyde (1.79, 13.18 mmoles) was then added. The mixture was refluxed for 4 hours. The reaction was quenched with cooled $H_2O$ (2 ml), extracted into ethylacetate (5 x 20 ml) and purified by column chromatography. $^1H$ NMR ($CDCl_3$) $\delta$ 7.2 (4H, m), 6.8 (4H,m), 6.5 (2H,s), 3.7 (6H,s); Mass Spectrum (FAB) m/e (M+1) 241.

Hydroxylated Metabolite of Compound IIIb (via Wittig Reaction)

(E)-3-Hydroxy-4,4'-dimethoxystilbene

To a stirred mixture of 4-methoxybenzyltriphenylphosphonium chloride (5.51g, 13mmol) and 4-methoxy-3-hydroxybenzaldehyde (2g, 13mmol) in $CH_2Cl_2$ was added a cooled aqueous solution of NaOH(62.5eq) in $H_2O$. The mixture was stirred at room temperature for 48h. The aqueous layer was then acidified to pH 5 and the precipitated solid was filtered and dried. Purification by silica gel chromatography using hexane/ethyl acetate

(1:1) gave the title E-trans product as a colourless solid 0.11g (3%): $^1$H NMR (DMSO) δ 9.3(1H, bs), 7.6(2H,d), 7.2(7H,m), 3.5(6H,s); Mass Spectrum (FAB) 257 (M+1).

Compound VII

(E)-1-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one (DMU-102)

To a stirred solution of 4-methoxybenzaldehyde (1.0g, 7.3 mmol) and 3,4,5-trimethoxyacetophenone (1.54 g, 7.3 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 h at room temperature, acidified with 2N HCl and extracted with ethyl acetate (3 x 30 ml). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent evaporated in vacuo. The product was purified by column chromatography followed by recrystallisation from methanol to afford the title compound as a pale yellow solid 1.22g (51%). $^1$H NMR ($CDCl_3$) δ 7.8 (1H, d), 7.6 (2H,m), 7.4 (1H,d), 7.3 (2H,d), 7.0 (2H,d), 3.9 (9H,s), 3.8 (3H,s). Mass Spectrum (FAB) m/e 329 (M+1).

Hydroxylated Metabolite of Compound VII

(E)-1-(3-Hydroxy-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one

To a stirred solution of 3-hydroxy-4-methoxybenzaldehyde (1.0g, 6.57 mmol) and 3,4,5-trimethoxyacetophenone (1.38g, 6.57 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 h at room temperature, acidified with 2N HCl, and extracted with ethyl acetate (3 x 30 ml). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent evaporated in vacuo. The product was purified by crystallisation from methanol (0.63g, 28% yield). $^1$H-NMR($CDCl_3$) δ 7.8 (1H, d), 7.3 (4H,m), 7.2 (1H,m), 6.9 (1H,d), 5.7 (1H,s), 3.9 (12H,s). Mass Spectrum (FAB) m/e 345 (M+1),

Compound XII (via Wittig Reaction)

(E)-3,4,5-Trimethoxy-4'-methoxystilbene (DMU-212)

To a stirred mixture of 4-methoxybenzyltriphenylphosphonium chloride (6.4g, 15.3 mmol) and 3,4,5-trimethoxybenzaldehyde (3g, 15.3 mmol) in $CH_2Cl_2$ was added a cooled aqueous solution of NaOH (62.5 eq) in $H_2O$. The mixture was stirred at room temperature for 48 hours. The organic phase was separated and the aqueous phase was washed with $CH_2Cl_2$. The organic phase was concentrated and the residue was recrystallised from ethanol to yield the title E-trans isomer. $^1$H NMR ($CDCl_3$) δ 8.7 (1H, d:), 8.2 (4H,m), 8.0 (2H,s), 5.2 (6H,s), 5.0 (6H,s). Mass Spectrum (FAB) 301 (M+1).

Compound VI

(Z)-3,4,5-Trimethoxy-4'-methoxystilbene (DMU-213)

The filtrate from the recrystallisation step following the preparation of Compound XII above was concentrated and purified by column chromatography using hexane/ethyl acetate (8:2) as eluant to give the title Z-cis isomer as a colourless solid 0.1g (yield 7%). $^1$H NMR ($CDCl_3$) δ 7.2 (2H, m), 6.8 (2H,m), 6.5 (4H,m), 3.9 (3H,s), 3.8 (3H,s), 3.7 (6H,s). Mass Spectrum (FAB) 301 (M+1).

Compound VIII

(E)-1-(4-Hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one (DMU-116)

To a stirred solution of 4-hydroxybenzaldehyde (1.0g, 8.19 mmol) and 3,4,5-trimethoxyacetophenone (1.72g, 8.19 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 hours at room temperature, acidified with 2N HCl and extracted with ethyl acetate (3 x 30 ml). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent evaporated in vacuo. The product was purified by column chromatography using hexane/ethyl acetate (7:3) as eluent to afford the title compound as a pale yellow solid (0.125g, 5%). $^1$H-NMR ($CDCl_3$) δ 7.8 (1H, d), 7.6 (2H,m), 7.4 (1H,s), 7.25 (2H,m), 6.9 (2H,d), 5.6 (1H,bs) 3.95 (9H,s). Mass Spectrum (FAB) m/e 315 (M+1).

Compound IX

(E)-1-(2,4-Dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one (DMU-132)

To a stirred solution of 2,4-dimethoxybenzaldehyde (1g, 6.00 mmol) and 3,4,5-trimethoxyacetophenone (1.27g, 6.00 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 hours at room temperature, acidified with 2N HCl and extracted with ethyl acetate (3 x 30 ml). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent evaporated in vacuo. The product was purified by recrystallisation from ethanol to afford the title compound as a pale yellow solid, 1.67g (78%). $^1$H-NMR ($CDCl_3$) $\delta$ 8.0 (1H, d), 7.5 (1H,d), 7.4 (1H,d), 7.2 (2H,s), 6.6 (1H,dd), 6.5 (1H,d), 3.9 (9H,d), 3.85 (3H,s), 3.83 (3H,s). Mass Spectrum (FAB) m/e 359 (M+1).

Compound X (DMU-129)

To a stirred solution of 6-methoxy-1-tetralone (1.28g, 7.3 mmol) and 3,4,5-trimethoxyacetophenone (1.54g, 7.3 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 hours at room temperature, acidified with 2N HCl and extracted with ethyl acetate (3 x 30 ml). The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent evaporated in vacuo. The product was purified by column chromatography, followed by recrystallisation from ethanol to afford the title compound 0.25g (9%). $^1$H NMR ($CDCl_3$) $\delta$ 7.4 (1H, s), 7.1-7.3 (5H,m), 3.9 (9H,s), 3.8 (3H,s), 1.6-2.3 (6H, complex m). Mass Spectrum (FAB) m/e 369 (M+1).

Compound XI (DMU-122)

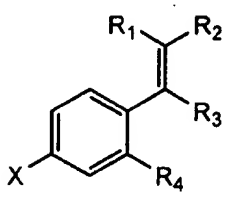
(E)-1-(4-Methoxyphenyl)-2-methyl-3-(3,4,5-trimethoxyphenyl)prop-1-en-3-one

To a stirred solution of 4-methoxybenzaldehyde (1.0g, 7.3 mmol) and 1-(3,4,5-trimethoxyphenyl)propan-1-one (1.64g, 7.3 mmol) in methanol (30 ml) was added a 50% w/v solution of aqueous NaOH (1 ml). The mixture was stirred for 24 hours at room temperature, acidified with 2N HCl and extracted with ethyl acetate (3 x 30 ml). The

combined organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The product was purified by column chromatography using hexane/ethylacetate (4:1) as eluant, followed by recrystallisation from ethanol to afford the title compound as a pale yellow solid, 0.36g (14%). $^1$H-NMR ($CDCl_3$) δ 7.8 (1H,s), 7.6 (2H,m), 7.3 (2H,d), 7.0 (2H,d), 3.9 (9H,s), 3.8 (3H,s), 2.3 (3H,s). Mass Spectrum (FAB) m/e 343 (M+1).

# CLAIMS

1. A prodrug activated by enzymatic aromatic hydroxylation and having the formula (I):




wherein:

X = H, OH or OMe;

$R_1$ = H, $C_{1-4}$ lower alkyl, CN or Ar;

$R_2$ = H, CN, $CONH_2$, $CSNH_2$, COAr or Ar; and

Ar = phenyl, pyridyl or substituted aryl;

and:

$R_3$ = H or $C_{1-4}$ lower alkyl; and

$R_4$ = H, OH or OMe;

or:

$R_3$,$R_4$ = $(CH_2)_n$, n=2, 3 or 4

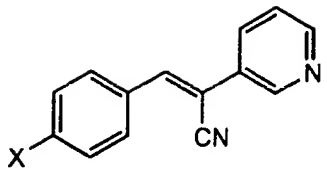
2. A prodrug according to claim 1, being an anti-tumour prodrug.

3. A prodrug according to claim 1, being hydroxylated by CYP1B1.

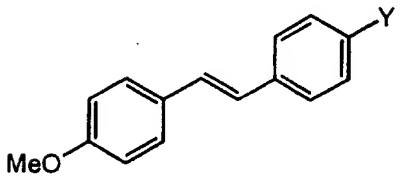
4. A prodrug according to claim 1, X being a hydroxy or methoxy group.

5. A prodrug according to any one of claims 1-4, having the formula of any one of formulae (II) - (V):

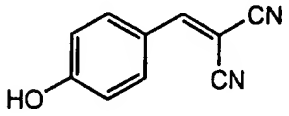
(II):



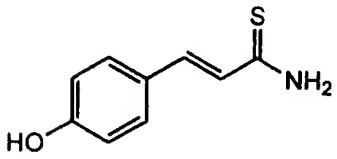

(III):




(IV):



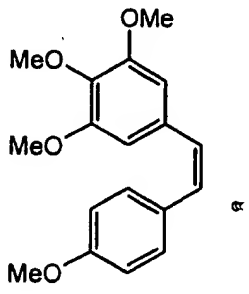

(V):



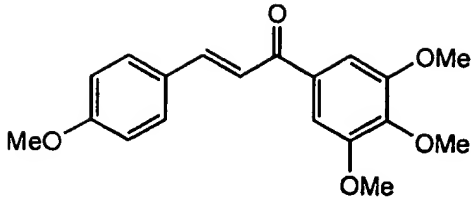

wherein X is selected from either one of the groupof OMe and OH, and Y is selected from either one of the group of $NO_2$ and OMe.

6. A prodrug acording to any one of claims 1-4, having the formula of either one of formulae (VI) or (VII):

(VI):

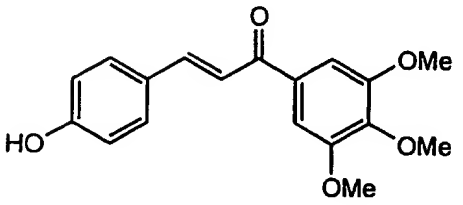


(VII):

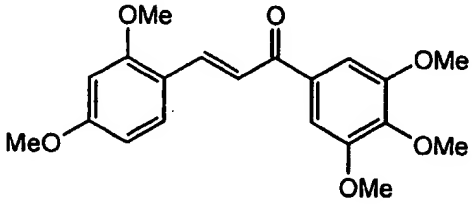



7. A prodrug according to any one of claims 1-4, having the formula of any one of formulae (VIII) - (XII):

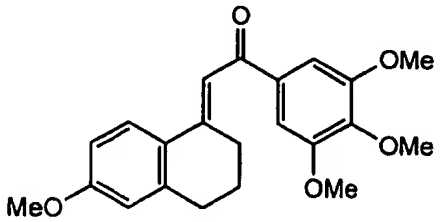
(VIII):



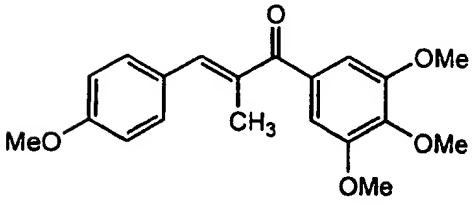

(IX):



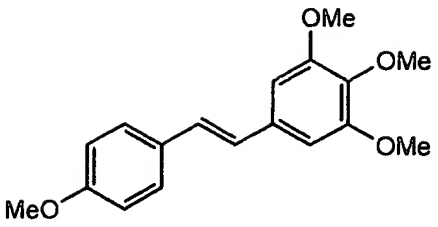

(X):




(XI):



(XII):




8. A prodrug according to claim 1 or 5, being a tyrosine kinase inhibitor in its hydroxylated state.

9. A prodrug according to claim 1, 6 or 7, being an antimitotic agent in its hydroxylated state.

10. A prodrug according to any one of the preceding claims for use in a method of treatment or diagnosis of the human or animal body.

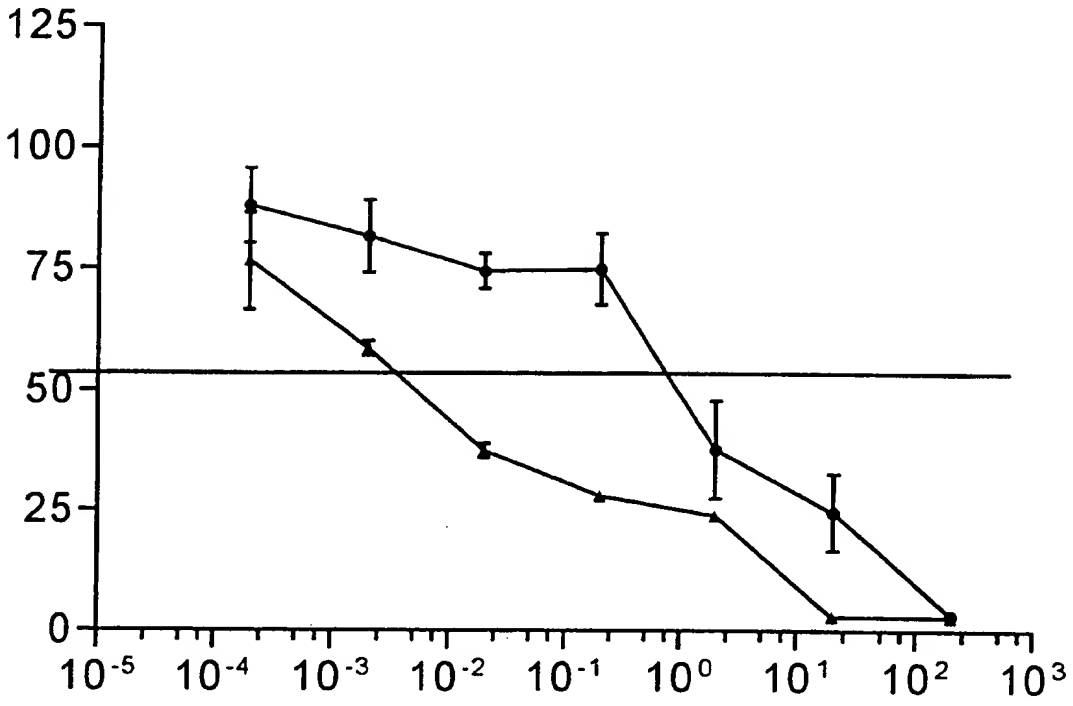
11. The use of a prodrug according to any one of claims 1-9 in the manufacture of a medicament for the treatment of a tumour.

12. A method of manufacture of a medicament for the treatment of a tumour, comprising the use of a prodrug according to any one of claims 1-9.

13. A method of treatment of a tumour in a patient, comprising administering to the patient a prodrug according to any one of claims 1-9.

14. The hydroxylated form of a prodrug according to any one of claims 1-9.

Figure 1



# INTERNATIONAL SEARCH REPORT

Into onal Application No
PCT/GB 99/00155

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C49/84 C07C43/215 C07C205/35 C07C255/36 C07D213/57
A61K31/09 A61K31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 430 062 A (MARK S. CUSHMANN ET AL.)<br>4 July 1995<br>see column 1, line 9 - column 3, line 16<br>see column 8, line 65 - line 69<br>see column 23, line 38 - column 26, line 47; claims; examples; tables I,II,V,VII-XI<br>--- | 1-14 |
| X | CUSHMAN M ET AL: "SYNTHESIS AND EVALUATION OF ANALOGUES OF (Z)-1-(4-METHOXYPHENYL)-2-(3,4,5-TRIMETHOXYPHENYL)ETHENE AS POTENTIAL CYTOTOXIC AND ANTIMITOTIC AGENTS"<br>JOURNAL OF MEDICINAL CHEMISTRY,<br>vol. 35, no. 12, 12 June 1992, pages 2293-2306, XP000571677<br>see tables I,II,IV<br>---<br>-/-- | 1-14 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 May 1999 | 04/06/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Zervas, B |

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# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 99/00155

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ' | Citation of document, with indication.where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GEORGE R. PETTIT ET AL.: "Antineoplastic Agents 322. Synthesis of Combretastin A-4 Produgs" ANTI-CANCER DRUG DESIGN, vol. 10, 1995, pages 299-309, XP002102893 see page 299, line 1 - page 301, line 17 see page 307, line 40 - page 308, line 12 --- | 1-14 |
| X | PATENT ABSTRACTS OF JAPAN vol. 096, no. 011, 29 November 1996 & JP 08 188546 A (KYOWA HAKKO KOGYO CO LTD), 23 July 1996 see abstract --- | 1-14 |
| X | EP 0 322 738 A (YISSUM RESEARCH DEVELOPMENT ) 5 July 1989 see page 3, line 1 - page 5, line 17; claims; examples 2-4,6,9 --- | 1-5,8-14 |
| X | SAJJAT HUSSOIN ET AL.: "Polyhydroxylated Phenylacrylic Acid Derivatives as New Anti-tumor Agents" JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 80, no. 5, May 1991, pages 416-418, XP002102894 WASHINGTON US see page 417, column 1, line 1 - page 418, column 2, line 38 --- | 1-5,8-14 |
| X | US 5 276 058 A (NIPPON HYPOX) 4 January 1994 see claims; examples; tables --- | 14 |
| X | PATENT ABSTRACTS OF JAPAN vol. 010, no. 245 (C-368), 22 August 1986 & JP 61 076433 A (TOYOBO CO LTD), 18 April 1986 see abstract --- | 1 |
| X | CHEMICAL ABSTRACTS, vol. 68, no. 5, 29 January 1968 Columbus, Ohio, US; abstract no. 21141r, G. PAPPALARDO ET AL.: "Relation between Structure and Antibacterial Action of Arylthioamides. II. Antibacterial Activity, Ultraviolet and Infrared Spectra, and Acid Hydrolysis of Aryl- and Arylvinylenethioamides" page 2014; column 2; XP002102896 see abstract & FARMACO, ED. SCI., vol. 22, no. 10, 1967, pages 808-820, --- -/-- | 1 |

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 99/00155

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | KOJI OHSUMI ET AL.: "Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure-Activity Relationships" JOURNAL OF MEDICINAL CHEMISTRY., vol. 41, no. 16, 30 July 1998, pages 3022-3032, XP002102895 WASHINGTON US see page 3025, column 1, line 4 - page 3028, column 1, line 2; table 1 --- | 1-14 |
| P,X | DUCKI S ET AL: "Potent antimitotic and cell growth inhibitory properties of substituted chalcones" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 8, no. 9, 1 May 1998, page 1051-1056 XP004137018 see tables 1 - 3, compounds 2a, 5a; figure 1 ----- | 1-14 |

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB 99/00155

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

.nformation on patent family members

Inte ional Application No
PCT/GB 99/00155

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5430062 A | 04-07-1995 | EP 0641301 A | 08-03-1995 |
| | | AU 4385293 A | 13-12-1993 |
| | | WO 9323357 A | 25-11-1993 |
| EP 322738 A | 05-07-1989 | AT 120955 T | 15-04-1995 |
| | | AU 632992 B | 21-01-1993 |
| | | AU 2736088 A | 29-06-1989 |
| | | CA 1334826 A | 21-03-1995 |
| | | DE 3853577 D | 18-05-1995 |
| | | DE 3853577 T | 31-08-1995 |
| | | EP 0614661 A | 14-09-1994 |
| | | ES 2073398 T | 16-08-1995 |
| | | JP 10279477 A | 20-10-1998 |
| | | JP 2138238 A | 28-05-1990 |
| | | JP 2806954 B | 30-09-1998 |
| | | US 5217999 A | 08-06-1993 |
| US 5276058 A | 04-01-1994 | EP 0629602 A | 21-12-1994 |
| | | DE 69313839 D | 16-10-1997 |
| | | DE 69313839 T | 19-02-1998 |